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Autor:	König, F. / Zwahlen, R. / Schaller, J.		
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Fig. 1: Immunohistochemical evidence of Factor VIII on confluent endothelial cell culture. Left: positiv, right, negativ control.

(Cytodex 3, Pharmacia) suspended in stirrer-bottles. A magnetic stirrer allowed modulation of the rotation time and speed. When the microcarriers were totally covered with EC (Fig. 2), they were rinsed in serum free medium, aliquoted into tubes; then were treated with C5a (1:250) or 10<sup>-9</sup> M platelet activating factor (PAF). Two tubes remained untreated, indicating the basal adherence values. After the treatment, the covered microcarriers were loaded in borosilicate glass columns and kept at 37°C. PMN were isolated from bovine whole blood containing the anticoagulant ACD (Acid Citrate Dextrose) in a 9:1 ratio. After centrifugation, the plasma layer, buffy coat and top half of the erythrocytes were discarded. The red cell fraction was then subjected to flash hypotonic lysis with cold distilled water, followed by reconstitution of isotonicity with hypertonic saline solution and centrifugation. Separation of neutrophils from eosinophils was achieved on a discontinuous Percoll gradient, separating the cells according to their density. The PMN pellet was then resuspended in a balanced salt solution and its concentration adjusted to 5x10 6 cells/ml. The PMN vitality was assessed by Trypan blue dye exclusion.



Fig. 2: Confluent endothelial cell culture on Cytodex microcarriers.

Adherence assay: The columns, loaded with the EC-covered microcarriers, were drained of their medium to the top of the microcarrierlayer; the PMN-suspension, untreated or treated (510 µM dexamethasone or 3.3 mM phenylbutazone, respectively) was then loaded on the columns. A sample of PMN-suspension was taken before and after passage through the columns, allowing the calculation of adherence. The results thus obtained gave values of 56% for basal adherence, while C5a stimulated cells presented an enhanced adherence of 110% of the control value. Incubation of PMN with dexamethasone reduced the adherence to 34%, and with phenylbutazone to 8% of the control value. EC stimulated with 10<sup>-9</sup> M PAF presented an adherence of 98% of the control. Preincubation of PMN with dexamethasone and phenylbutazone reduced those values to 39%, and 11% respectively. These results indicate clearly that the effects of antiinflammatory drugs can be reproduced in a physiological isolated system. This novel system is now being tested more extensively. It shoul help reducing the number of experimental animals used in assessing pharmacological and toxicological data on new drugs.

Institut of Veterinary Pathology, University of Berne, Switzerland

# BOVINE CARDIOMYOPATHY: PATHOMORPHOGENETIC AND BIOCHEMICAL STUDIES IN YEARLING STEERS

## F. König, R. Zwahlen, J. Schaller, U. Kämpfer, D. Roth, A. Tontis, H. Luginbühl

In Switzerland bovine cardiomyopathy (BCMP) is a serious problem of SimmentalxHolstein crossbred cattle, causing severe economic losses in the dairy industry. While the morphologic features of the advanced stages of this invariably fatal disease have been described in detail, information on development of lesions and on the pathogenesis of the disease still remains scant.

#### **Material and methods**

In the present study we analyzed organs from 172 yearling steers (group A: 78 crossbred steers, genetically disposed to BCMP; group

B: 70 crossbred steers, of genetic low risk for BCMP; and group C [controls]: 24 pure Simmental steers). None of the selected steers showed clinical signs of illness. The following methods were used for morphological, and in selected animals, for additional biochemical analyses: qualitative and semiquantitative randomized histologic evaluation of BCMP related lesions in heart, lung, liver and kidney; biochemical analysis of the myocardial amino-acid pattern in selected animals of our study population (12 controls and 3 animals with histologically confirmed myocardial degeneration and fibrosis) and, additionally, biochemical analysis of 6 adults (positive control) with clinically manifest BCMP by acid hydrolysis and HPLC.

To enable statistical evaluation, the following histologic criteria were chosen: presence or absence of cardiomyocyte necrosis and degree of peri- and endomysial myocardial fibrosis; presence or absence of vascular changes in small pulmonary veins; presence or absence of foci of interstitial nephritis; presence or absence of hepatic centrolobular congestion in connection with hepatocyte degeneration.

#### **Results and discussion**

Cardiomyocyte necrosis was found on 5.8% of histological slides of control animals, on 10.3% in B animals, and on 15.1% of A animals (C vs. A: p < 0.01; B vs. A: p < 0.05). When 3 or more out of the 5 slides from each heart showed necrosis, the animal was classified as «necrosis positive» (Np): 9 A, 5 B and no C animals fell into this category. 7 of these 14 animals (4 A and 3 B) presented additionally distinct myocardial fibrosis, a finding not recognized in animals without significant cardiomyocyte necrosis. Sporadic changes in other organs (lung, kidney, liver) were rare and there was no evidence of statistical differences between the three groups (p > 0.05).

Biochemical myocardial amino-acid analysis revealed the following amino-acid ratios:

Amino-acid ratios	Controls (N=12)	Confirmed cases of BCMP (N=6)	Steers with fibrosis and necrosis (N=3)
Lys/OH-Pro	$18.56\pm2.98$	$2.46 \pm 0.59^{*}$	$5.03 \pm 1.22*$
His/OH-Pro	$4.69\pm0.76$	$0.73 \pm 0.15*$	$1.66\pm0.45^{*}$

\*  $p \pm 0.001$  when values are compared with respective control values

The study population consisted of animals kept in comparable environmental conditions, they only differed in their ancestry. The identified cardiomyocyte necrosis (Np) could represent the first visible lesion of BMCP, because necrosis without fibrosis could be observed, whilst fibrosis without necrosis did not occur. Degeneration of cells attracts macrophages and also induces fibroblast proliferation. This could partially explain the observed increase of collagen fibers. Additionally, loss of contractile units through cardiomyocyte necrosis may result in hypertrophy of remaining cardiomyocytes in order to maintain an adequate heart performance. As the disease progresses, heart failure develops and wall tensions then rise through the increasing ventricular dilatation, so provoking an additional increase in myocardial collagen. The lesions found in liver and lung of advanced cases most probably are secondary changes resulting from congestive heart failure. The result of myocardial amino-acid analysis discloses a possible approach to a biochemical test for detecting early stages of the disease. Further studies are needed to confirm these findings, and to expand biochemical analysis to other domains such as finding markers for the disease in body fluids, with the aim to establish a simple and rapid screening test for this fatal and costly disease.

#### Veterinär-Medizinische Klinik, Universität Zürich

#### **BIOLOGY OF THE FELINE IMMUNODEFICIENCY VIRUS**

#### H. Lutz

Feline immunodeficiency virus (FIV), a lentivirus, was first described in 1987 in cats with clinical symptoms of immunodeficiency. Since its discovery, it has been detected all over the world and it has been cloned and sequenced. In its structure it is very similar to the lentiviruses of other species. Major components of the virus are gag proteins with apparent molecular weights of 10 000, 15 000 and 24 000 daltons, and components of the envelope with 43 000 and 130 000 daltons. Immunologically FIV is only distantly related with lentiviruses of other species. An exception is equine infectious anemia virus (EIAV); antibodies to FIV p24 strongly cross-react with EIAV p26.

In nature FIV-infection is mainly transmitted by bites. In most cases kittens born to FIV-infected queens are not infected. FIV-infection is routinely diagnosed by the detection of antibodies, usually by ELISA's or by immunofluorescence assay. Up to 15% of FIV-infected sick cats are antibody-negative. Therefore the frequency and importance of FIV-infection in sick cats may be underestimated.

The overall FIV-infection rate in different countries varies greatly. Countries with a low rate of around 1-3% are Switzerland, Germany and the Netherlands; in many other countries the frequency is higher; the maximum rate being in Japan (over 25%). FIV-infection affects

mostly male cats with access to outdoors. The average age of FIV-infected sick cats in the so-called AIDS-phase of the disease was determined to be around 5 years.

The major clinical symptoms found in FIV-infected cats include fever, malaise, weight loss, chronic infections of the oral cavity and the upper respiratory tract, conjunctivitis and bacterial infections of the skin and ears. However, as similar symptoms are also observed in animals with other viral infections, FIV-infection can not reliably be diagnosed by the clinical symptoms alone. At present, there is no etiologic therapy available. Therapy consists of symptomatic treatment of secondary infections.

Experimental infection with FIV of specific pathogen free (SPF) cats leads to neutropenia, intermittent fever and lymphadenopathy for a period of several weeks starting 3 to 5 weeks p.i. After this initial phase cats usually recover and remain free of clinical symptoms for months and even years. The AIDS-phase of the disease can only rarely be observed under SPF-conditions although after prolonged infection the immune system of the cats is severely impaired: In a recent study it was shown that after longterm experimental FIV-infection (>26 months) SPF cats exhibited a depressed humoral immune response to a T-dependent, synthetic polypeptide. The cats also exhibited a decrease in the number and percentage of CD4+ lym-